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INDUSTRIAL ANALYTICAL APPLICATIONS OF RAPID ION-EXCHANGE SEPARATIONS OF WEAK ORGANIC ACIDS

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SUMMARY

Rapid ion-exchange chromatography has been adapted to the separation of industrial mixtures of weak organic acids. Minor impurities can be determined with a sensitivity of 0.1%. A methanol solution of the sample is injected directly into a 2.8 × 500 mm acetate form ion-exchange column. Using a specially designed gradient elution apparatus separation is achieved with an acetic acid–methanol gradient. The eluate proceeds through a flow cell mounted in an ultraviolet spectrophotometer. Response of the spectrophotometer is monitored on a strip-chart recorder. Concentrations are calculated by comparing peak areas with those of standards. Separations are generally produced in thirty to sixty minutes. Products analyzed by this technique include: 2,4-dichlorophenol, 2,4,6-trichlorophenol, 2,2′, 6,6′-tetrabromobisphenol A, 2,6-dimethyl-4-pyridinol, 3,4′,5-tribromosalicylanilide, and 3,5,6-trichloro-2-pyridinol.

INTRODUCTION

In the past two years there has been rapid growth in the area of liquid chromatography. Various commercial instruments have been designed and placed on the market. However, a very limited number of publications have appeared illustrating the application of rapid liquid chromatography to the analysis of industrial products. Generally the authors have been concerned with the separation of components present in near-equal concentrations. It was the purpose of this work to develop a system that would be competitive with gas chromatography in respect to such operations as sample introduction, separation time, and column regeneration; and the application of this technology to the analysis of commercial products.

LOGIE[1] indicated the usefulness of acetate form resins for the separation of chlorophenols. This work was extended by SKELLY[2] who employed a gradient elution which gave increased resolution. Various automated systems have been developed for the separation of weak organic acids. These have employed either ion-exchange[3] or partition[4] chromatography for the mode of separation. DAVIES *et al.*[5] made a comprehensive study of the ion-exchange characteristics of a number of organic

acids. An unusual, though complex, gradient elution apparatus was recently reported by STEHLIK[6].

EXPERIMENTAL

Reagents

Methanol, ACS grade without further purification.

Glacial acetic acid.

Organic standards. All organic standards were prepared in The Dow Chemical Company Research Laboratories. Structure determination was accomplished by elemental analysis and rigorous IR, NMR, and MS examination.

Acetic acid–methanol solutions. All eluent solutions were prepared on a volume (v/v) basis.

Ion-exchange resin, AG1-X2, acetate form, 200–400 mesh, Bio-Rad Laboratories, 32nd and Griffin, Richmond, Calif. 94804. A portion of the resin was placed in a $\frac{1}{2}$-in.-diameter column and washed with 100 ml of glacial acetic acid. Following extrusion from the column, the resin was washed three times with methanol by decantation. It was then stored under methanol in a closed container until used.

Apparatus

Syringe, 10 μl, No. 701-N, The Hamilton Co., Whittier, Calif.

Pump, Milton Roy Instrument miniPump®, Catalog No. 196-31, St. Petersburg, Fla.

Chromatography columns and fittings. The 2.8 × 500 mm columns and fittings were obtained from Chromatronix Inc., 2743 Eight Street, Berkeley, Calif. 94710.

Column septum. A 6.5 mm disc was cut from Type W silicone rubber, Catalog No. 15414, Applied Science Laboratories Inc., State College, Pa. 16801.

Column monitoring unit. The basic unit consisted of a Gilford Model 222 power supply, Gilford Instrument Co., 132 Artino Street, Oberlin, Ohio 44074. This was used in conjunction with a Beckman model DU monochromator and Sargent recorder, Model SRG, Catalog No. X574180-15 with chart speeds of 3, 6, and 12 in./h; Sargent-Welch Scientific Co., 7300 North Linden Avenue, Skokie, Ill. 60076.

Flow cells. 1-cm path length optical cells with bubble traps (Gilford Catalog No. 203A) were used for all measurements.

Connections. $\frac{1}{8}$-in.-I.D. Teflon® tubing was connected to the inlet side of the pump with a Swagelok fitting (Crawford Fitting Co., 884 East 140 St., Cleveland, Ohio). On the outlet side, $\frac{1}{16}$-in.-I.D. Teflon tubing was connected to the pump with a Swagelok fitting. The same size tubing was used from the column to the flow cell and from the flow cell to waste.

Stepwise gradient cell. This unit is illustrated in Fig. 1. 1-mm capillary tubing was used throughout. Stainless-steel 0.072-in.-O.D. tubing was attached at the various inlet and outlet points with the exception of the methanol reservoir which had 0.125-in.-O.D. tubing. All two- and three-way stopcocks were of Teflon plastic construction. Mixing flasks were 38 × 140 mm with 29/42 S/T joints. 40 ml of methanol was added to each flask and a calibration line was marked on the glass. Joint seals were made with 29/42 Teflon sleeves.

Continuous gradient cell. Similar dimension glassware and related fittings were

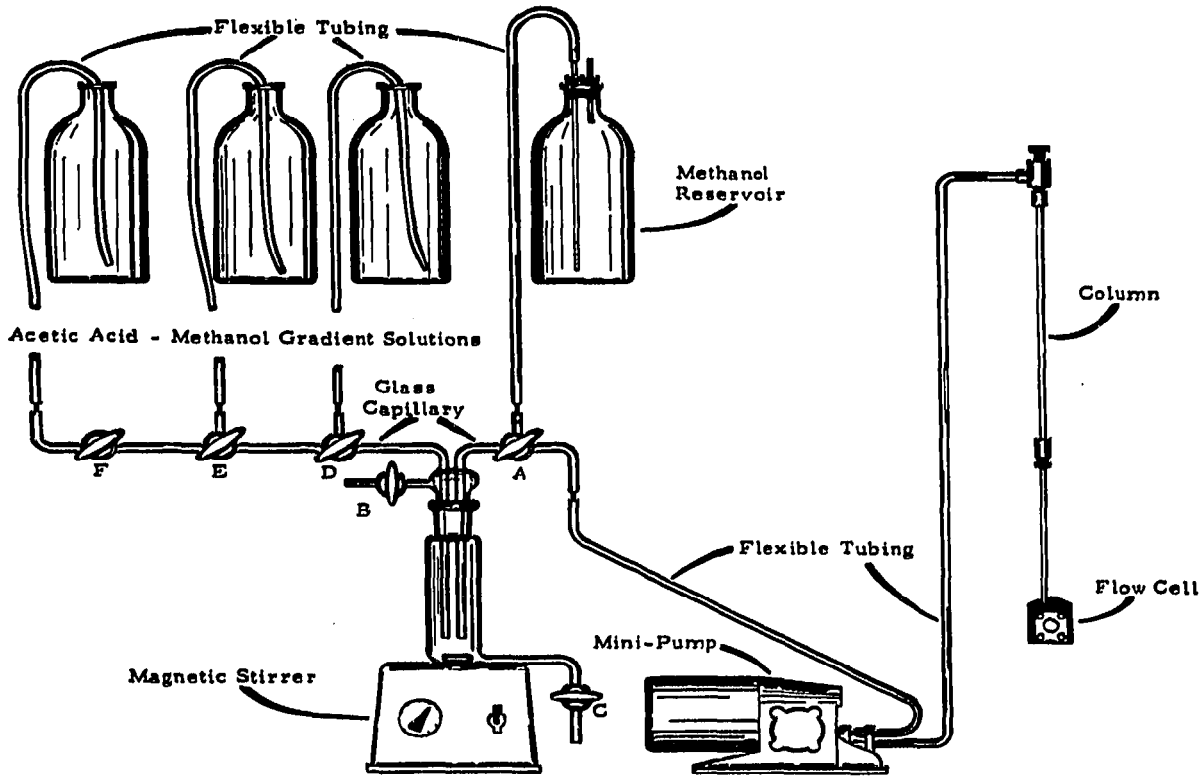

Fig. 1. Stepwise gradient cell.

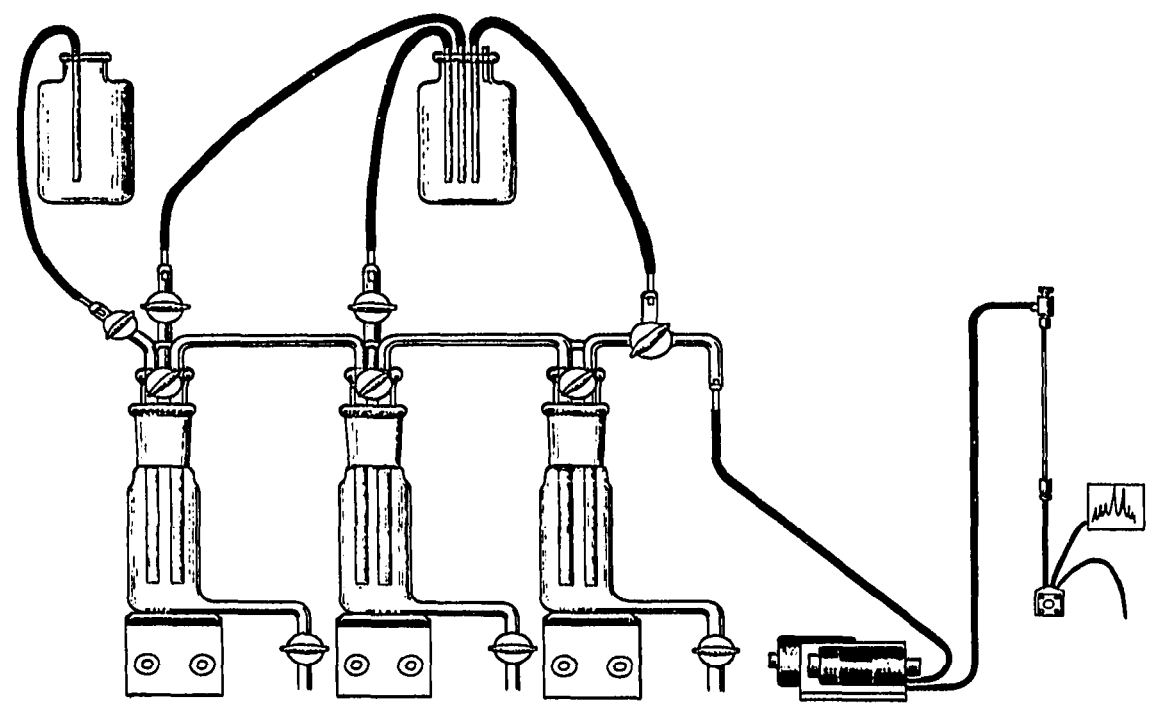

Fig. 2. Continuous gradient cell.

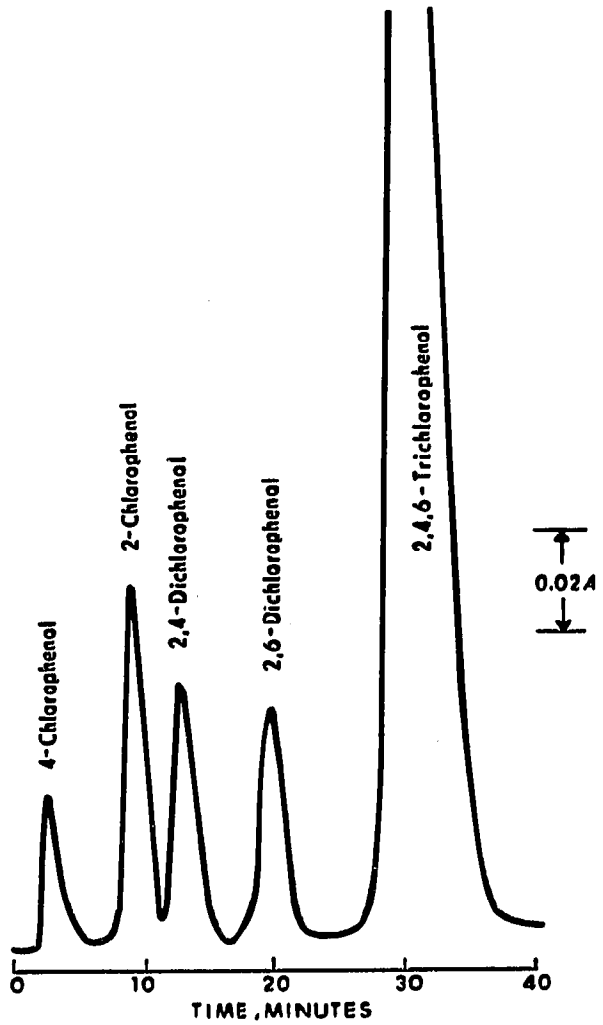

Fig. 3. Separation of 10 μl of a methanol solution containing 0.06% 4-chlorophenol, 0.13% 2-chlorophenol, 0.15% 2,4-dichlorophenol, 0.11% 2,6-dichlorophenol, and 2.52% 2,4,6-trichlorophenol on 2.8 × 500 mm AG1-X2 ion-exchange resin with 5% acetic acid–methanol, continuous gradient at 280 nm and a flow rate of 2.4 ml/min.

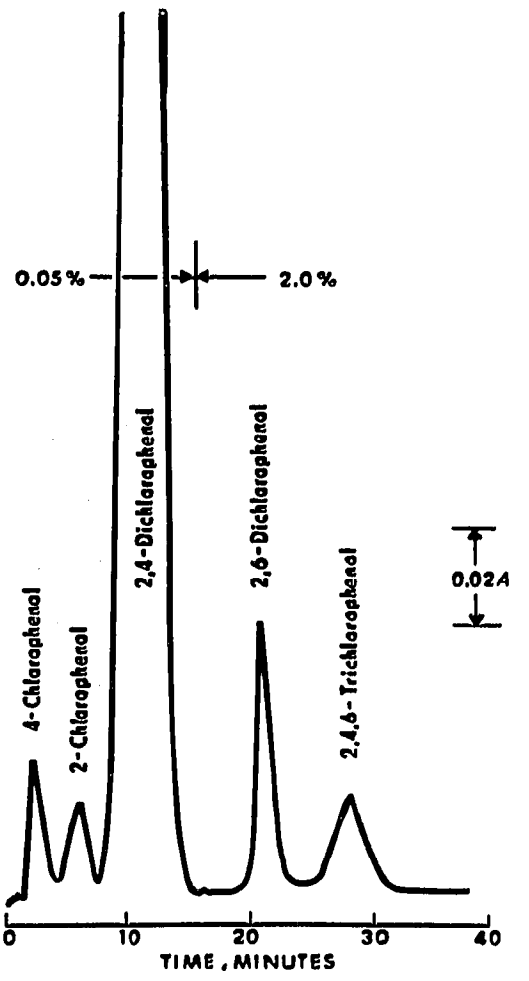

Fig. 4. Separation of 10 μl of a methanol solution containing 0.05% 4-chlorophenol, 0.04% 2-chlorophenol, 0.11% 2,6-dichlorophenol, 0.12% 2,4,6-trichlorophenol, and 3.0% 2,4-dichlorophenol on 2.8 × 500 mm AG1-X2 ion-exchange resin with a stepwise gradient of 0.05% and 2.0% acetic acid–methanol at 280 nm and a flow rate of 2.4 ml/min.

used in this unit which is illustrated in Fig. 2. A reinforcing glass rod was welded between the mixing chambers to give the apparatus added rigidity.

Column preparation

A plug of pyrex wool was placed at the bottom of the 2.8 × 500 mm tube. The membrane in the bed support disc was removed and the disc was replaced at the base of the column. Excessive back pressure was exerted by the membrane; therefore, the pyrex wool was used to hold the resin in place. A methanol slurry of the AG1-X2 resin was injected into the chromatography column through an 18-in. section of $\frac{1}{8}$-in.-I.D. Teflon tubing using a 10-ml hypodermic syringe and a luer lok

adapter. The luer lok adapter was removed and the final packing was accomplished with a flow of methanol under pump pressure.

Sample preparation

For a given analysis, 0.25–1.0 g samples are weighed into a 10-ml volumetric flask. The solid is dissolved and diluted to volume with methanol. Standard mixtures were prepared in a similar manner. Milligram amounts of the individual impurities were weighed into a 10-ml volumetric flask. 0.25-1 g of the major component was added to the flask. The sample was dissolved and diluted as before.

Procedure

The mixing flask was filled from the methanol reservoir with 40 ml of methanol through stopcock A, with drain stopcock C closed and vent stopcock D open. Eluent containers were filled with the desired concentration of acetic acid in methanol. The pump was turned on and methanol allowed to flow through the column. After select-

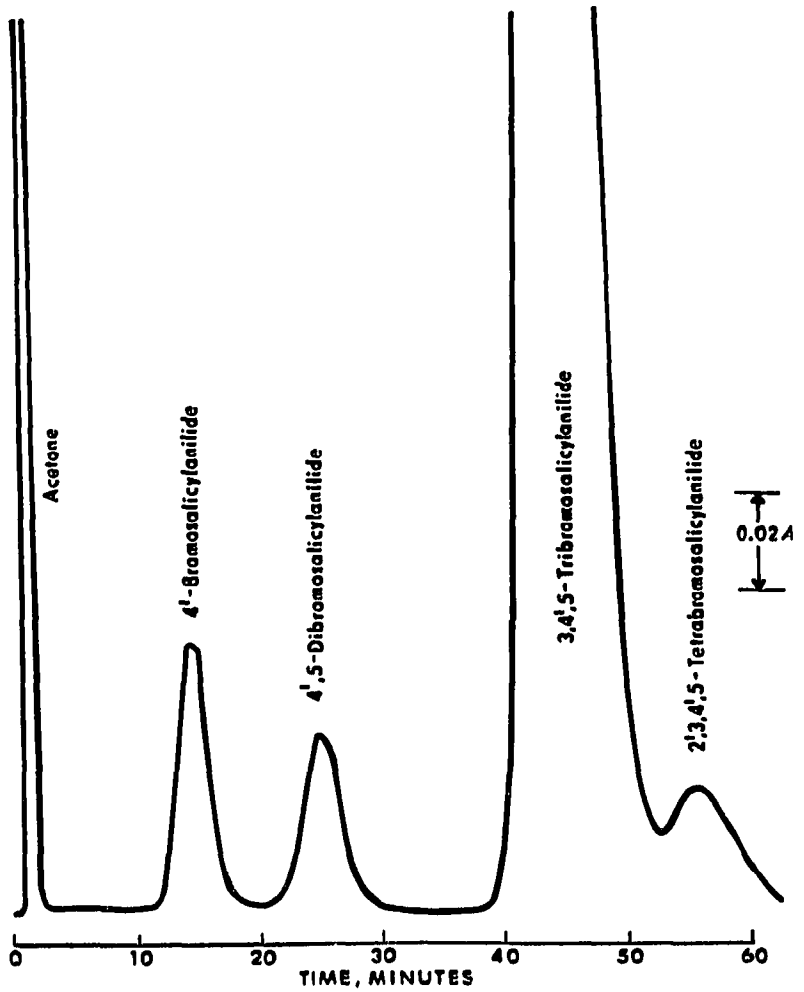

Fig. 5. Separation of 10 μl of an acetone solution containing 0.05% 4'-bromosalicylanilide, 0.05% 4',5-dibromosalicylanilide, 0.16% 2',3,4',5-tetrabromosalicylanilide, and 2.6% 3,4',5-tribromosalicylanilide on 2.8 × 500 mm AG1-X2 ion-exchange resin with a continuous gradient of 100% glacial acetic acid at 280 nm and a flow rate of 2.4 ml/min.

ing the appropriate wavelength, the instrument was nulled using the slit control knob and the null indicator. 10 μl of sample solution was injected directly into the resin through the silicone rubber septum. Vent stopcock D was closed, the magnetic stirrer activated and stopcocks D, E, or F were turned to the appropriate positions to allow the acetic acid–methanol solution to flow into the mixing flask. The eluent proceeds through the ion-exchange column and into the flow cell. Response of the spectrophotometer is monitored on a recorder.

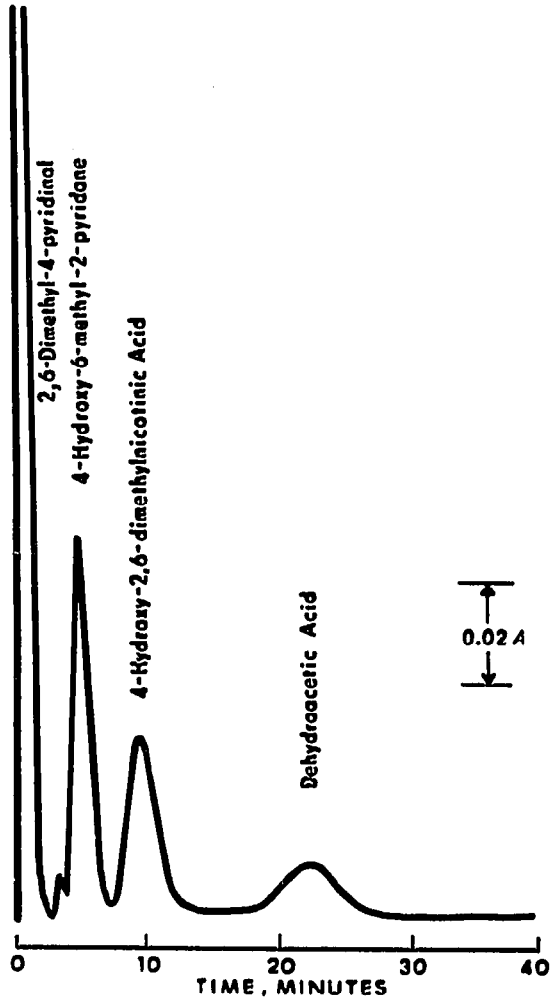

Fig. 6. Separation of 10 μl of a methanol solution containing 0.10% 4-hydroxy-2,6-dimethylnicotinic acid, 0.10% dehydroacetic acid, 0.05% 4-hydroxy-6-methyl-2-pyridone, and 5.0% 2,6-dimethyl-4-pyridinol on 2.8 × 500 mm AG1-X2 ion-exchange resin with a stepwise gradient elution of 1.0% acetic acid–methanol at 270 nm with a flow rate of 2.4 ml/min.

Concentration of the impurities was determined by measuring the areas in the resulting chromatogram and comparing these with standards. When the chromatogram was complete, stopcock A was turned to the methanol-wash position in preparation for the next run. Dual pumps, columns and gradient units make it possible to monitor one column while the other one is undergoing a wash cycle.

RESULTS AND DISCUSSION

Chromatograms for the separation of several industrial materials are given in Figs. 3–8. The components contained in the respective products may not necessarily be the same as that found in various production materials. This composition will be dependent upon the method of synthesis and the degree of purification.

The choice of elution system will depend on the pK range of the acid mixture to be separated. If the mixture contains acids having a wide range of pK values,

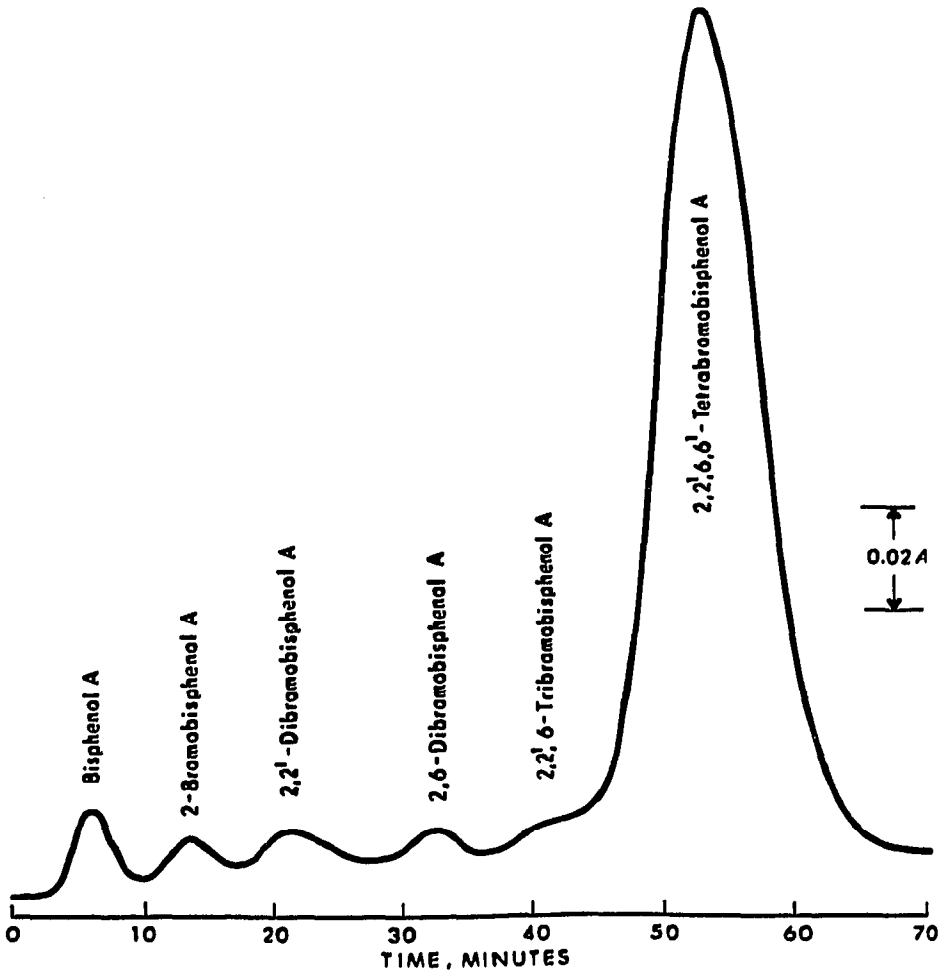

Fig. 7. Separation of 5 μl of a methanol solution containing 0.10% bisphenol A, 0.07% 2-bromobisphenol A, 0.10% 2,2'-dibromobisphenol A, 0.10% 2,6-dibromobisphenol A, 0.10% 2,2',6-tribromobisphenol A, and 5% 2,2',6,6'-tetrabromobisphenol A on 2.8 × 500 mm AG1-X2 ion-exchange resin with a continuous gradient elution of 1.0% acetic acid–methanol at 285 nm and a flow rate of 2.4 ml/min.

two choices of elution systems are available. Either a two or three step gradient elution using the stepwise unit may be used. Or, the continuous gradient cell may be preferred. The elution profile as obtained for the two units is illustrated in Fig. 9. 50% acetic acid–methanol was led into the mixing flask containing 40 ml of methanol. The column eluate was monitored at 256 nm. In the stepwise unit there is a rapid

buildup in the acetic acid concentration while the continuous cell allows a more gradual increase. Different sizes of mixing flasks may be substituted to change the gradient profile.

Neutral and basic compounds are not exchanged by the resin, but proceed directly into the eluate. If a mixture contains a neutral component as a minor impurity, the gradient can be started immediately following sample injection without causing this impurity and any weakly acidic impurities from emerging together. There is sufficient methanol in the column dead volume and tubing so that the effect of the gradient is delayed. However, if the major component is neutral, it may be necessary to elute this material with methanol before the gradient is started.

A sample solution that is acid by its very nature, or contains mineral acid, may require neutralization prior to introduction on the resin bed. Weakly acidic components may be eluted prematurely from this acid environment. In most in-

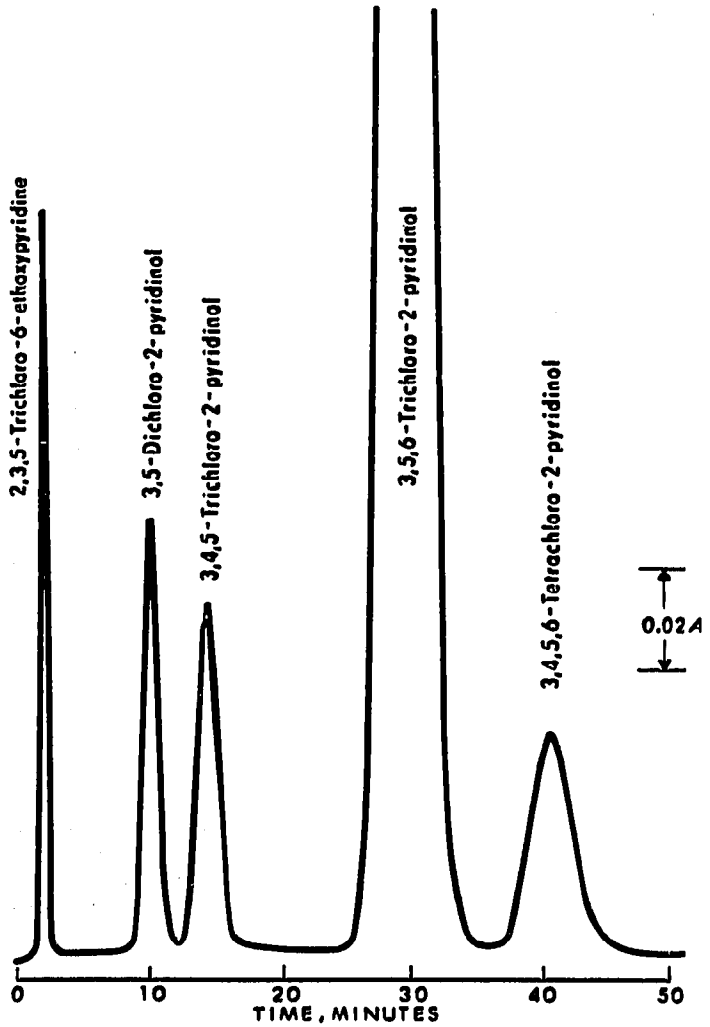

Fig. 8. Separation of 10 μl of a methanol solution containing 0.09% 2,3,5-trichloro-6-ethoxypyridine, 0.07% 3,5-dichloro-2-pyridinol, 0.09% 3,4,5-trichloro-2-pyridinol, 0.17% 3,4,5,6-tetrachloro-2-pyridinol, and 2.6% 3,5,6-trichloro-2-pyridinol on 2.8 × 500 mm AG1-X2 ion-exchange resin with a stepwise gradient elution of 100% glacial acetic acid at 310 nm and a flow rate of 2.4 ml/min.

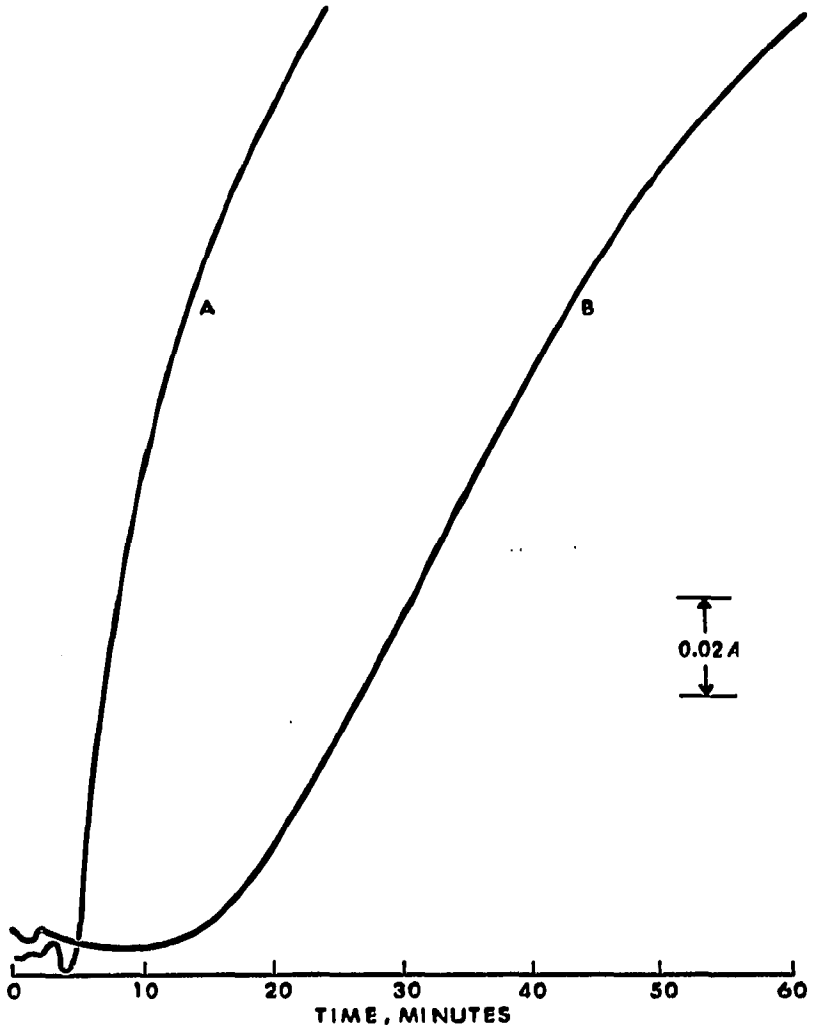

Fig. 9. Elution profile obtained by the stepwise gradient cell, A, and the continuous gradient cell, B, with 50% acetic acid–methanol monitored at 256 nm.

stances, with the use of μl sample sizes, the effect of the acidity is diluted out such that this does not present a problem.

Sensitivity of detection for the minor impurities is generally in the range of 0.1% relative to the major component. This will depend on how large a sample can be injected and on the degree of resolution for the components. On an absolute basis with compounds that have average UV sensitivity, 1 μg can be observed. Compounds that are eluted with difficulty and give long low peaks, will have a much higher limit of detection.

If the sample to be analyzed contains a diverse range of UV-absorbing species, a wavelength is chosen such that the components have near-equal sensitivity. Another factor that will influence this choice will be the relative concentrations of the impurities. If the sample contains a high concentration of one impurity and lesser amounts of others, it may be desirable to select a wavelength where the major impurity is insensitive and the minor impurities are extremely sensitive.

Samples that are sparingly soluble in methanol may also be analyzed by this

technique. Dissolution may be achieved with any neutral solvent. This will include UV radiation absorbing solvents. The UV-absorbing solvent will emerge with the solvent front and show up on the chromatogram as a sharp peak. Any neutral components present in the sample would be masked by the solvent. The separation of the brominated salicylanilides shown in Fig. 5 illustrates the use of a UV-absorbing solvent.

If the sample to be analyzed contains two neutral species, and they have different UV spectra, they can be analyzed by this technique. Duplicate injections are made in sequence with the spectrophotometer set at two different wavelengths. By making the appropriate calibrations and solving simultaneous equations the respective concentrations can be calculated.

Impurity concentrations are determined by either measuring peak height or peak area. Generally, the concentration of neutral and basic impurities, those which come through with the solvent front, are calculated from peak height measurements, while those compounds eluted with the gradient are calculated from peak areas. Peak heights and peak areas were found to obey Beer's law over the full scale absorbance range.

The use of special sample injection valve (Chromatronix Inc., SV-8031) allows the introduction of sample solutions above the microliter level. In fact, the limit is that of practicality. This makes it possible to concentrate on the resin weak acids from dilute solutions. Elution is then carried out in the normal manner. Chloroform extracts of aqueous solutions may be processed in this manner.

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